



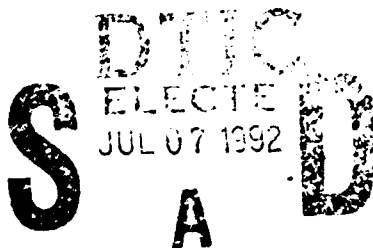
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June 1992

**NCEL**An Investigation Conducted by  
M. K. Han, R. E. Wyza, and  
R. F. Olfenbuttel**Contract Report**BATTELLE  
Columbus Division

# IN SITU GENERATION OF OXYGEN BY ELECTROLYSIS AND THE ELECTROCHEMICAL EFFECTS ON MICROORGANISMS' POPLUATION

**Abstract** The objective of this program was to quantitatively assess the effectiveness of in situ electrolysis of groundwater on the growth of soil microorganisms. The electrolysis method was used to supply oxygen to soil microorganisms to enhance bioremediation of sites that are contaminated with hydrocarbons. Electrochemical tests were performed with two different types of soils in a 10-gallon glass container. Experimental variables examined included driving voltage, current, and electrode spacing. Stainless steel mesh was used for both the anode and the cathode. Depending on the soil, a sustained supply of dissolved oxygen up to 16 ppm was achieved. The population of soil microorganisms increased with increasing the oxygen content in the groundwater. A slight decrease in the population was seen at the cathode, which might have been due to an increase in the pH at the cathode.



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# METRIC CONVERSION FACTORS

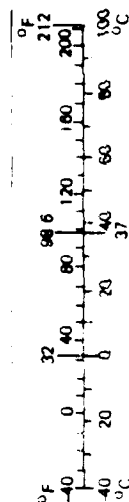


## Approximate Conversions to Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
<b>LENGTH</b>				
in	inches	2.5	centimeters	cm
ft	feet	30	centimeters	cm
yd	yards	0.9	meters	m
mi	miles	1.6	kilometers	km
<b>AREA</b>				
in <sup>2</sup>	square inches	6.5	square centimeters	cm <sup>2</sup>
ft <sup>2</sup>	square feet	0.09	square meters	m <sup>2</sup>
yd <sup>2</sup>	square yards	0.8	square meters	m <sup>2</sup>
mi <sup>2</sup>	square miles	2.6	square kilometers	km <sup>2</sup>
	acres	0.4	hectares	ha
<b>MASS (weight)</b>				
oz	ounces	28	grams	g
lb	pounds	0.45	kilograms	kg
	short tons (2,000 lb)	0.9	tonnes	t
<b>VOLUME</b>				
tsp	teaspoons	5	milliliters	ml
Tbsp	tablespoons	15	milliliters	ml
fl oz	fluid ounces	30	milliliters	ml
c	cups	0.24	liters	l
pt	pints	0.47	liters	l
qt	quarts	0.95	liters	l
gal	gallons	3.8	liters	l
ft <sup>3</sup>	cubic feet	0.03	cubic meters	m <sup>3</sup>
yd <sup>3</sup>	cubic yards	0.76	cubic meters	m <sup>3</sup>
<b>TEMPERATURE (exact)</b>				
°F	Fahrenheit temperature	5/9 (after subtracting 32)	Celsius temperature	°C

## Approximate Conversions from Metric Measures

When You Know	Multiply by	To Find	Symbol
<b>LENGTH</b>			
millimeters	0.04	inches	in
centimeters	0.4	inches	in
meters	3.3	feet	ft
kilometers	1.1	yard	yd
	0.6	miles	mi
<b>AREA</b>			
square centimeters	0.16	square inches	in <sup>2</sup>
square meters	1.2	square yards	yd <sup>2</sup>
square kilometers	0.4	square miles	mi <sup>2</sup>
hectares (10,000 m <sup>2</sup> )	2.5	acres	
<b>MASS (weight)</b>			
grams	0.035	ounces	oz
kilograms	2.2	pounds	lb
tonnes (1,000 kg)	1.1	short tons	
<b>VOLUME</b>			
milliliters	9.03	fluid ounces	fl oz
liters	2.1	pints	pt
liters	1.06	quarts	qt
liters	0.26	gallons	gal
cubic meters	35	cubic feet	ft <sup>3</sup>
cubic meters	1.3	cubic yards	yd <sup>3</sup>
<b>TEMPERATURE (exact)</b>			
Celsius temperature	9/5 (then add 32)	Fahrenheit temperature	°F



\* For 2.54 (exact). For other exact conversions and more detailed tables, see NBS Mon. Publ. 286 Units of Weights and Measures. Price \$2.25. SD Catalog No. C13.10.286

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## INTRODUCTION

The U.S. Navy has many sites contaminated with hydrocarbons that require cleanup. In-situ biodegradation of these contaminants is an emerging technology for remediation of the contaminated sites. The most significant obstacle to overcome in enhancing the in-situ biodegradation of organic compounds is the supply of oxygen to the microorganisms responsible for the degradation of hydrocarbons. For aerobic microorganisms, oxygen is required to sustain the metabolic processes they use to destroy organic contaminants.

Current technology for enhancing groundwater oxygen content is primarily based on the addition (injection) of hydrogen peroxide to the soil. Due to the rapid breakdown of hydrogen peroxide, however, this technique has not been proved to be as successful as desired. Experience at field sites indicates that rapid decomposition of hydrogen peroxide may preclude real world transport of higher levels of oxygen into a contaminated soil/aquifer.

Naval Civil Engineering Laboratory (NCEL) believes that in-situ electrolysis of groundwater may be a more effective means of delivering oxygen to the microorganisms in the soil. NCEL, therefore, has a requirement for a laboratory with experience in in-situ electrochemistry, microbial degradation, and in-situ decontamination to design and conduct an investigation into the feasibility of enhancing biodegradation of hydrocarbons by in-situ generation of oxygen by electrolysis.

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Battelle was contracted by NCEL, through the Army Research Office, to conduct a laboratory investigation to assess the feasibility of a field technique based on in-situ generation of oxygen.

### **OBJECTIVES**

The overall objective of this research program is to evaluate, on-site, the effectiveness of in-situ generation of oxygen by electrolysis and of the resulting enhancement of biodegradation of hydrocarbon contaminants.

The immediate objective of the initial phase of the program was to determine field-specific design parameters and to quantitatively assess the effectiveness of the method on the growth of aerobic microorganisms.

### **EXPERIMENTAL**

Electrochemical tests were performed in the laboratory using soils in a glass tank having the dimensions of 20-inches wide, 10-inches deep, and 10-inches high. The purpose of these electrochemical tests was to determine the dissolved oxygen contents in groundwater under various experimental conditions. Two types of soils were used in these tests: a sand/bentonite mixture and the soil supplied by NCEL from a site at Fallon, Nevada. Two counts of microorganisms were performed with the Fallon soil, before and after an electrolysis test, and the changes in the population of four types of microorganisms were measured.

#### **Experimental Apparatus**

A schematic drawing of the experimental apparatus used is shown in Figure 1. A glass container that was 20-inches wide, 10-inches deep, and 10-inches high was used as the soil cell. The depth of the soil sample was typically 7 inches. Tap water was used after compressed air had been bubbled through it for at least 24 hours prior to its flow through the soil layer. Stainless steel mesh electrodes were used for both the anode and the cathode.

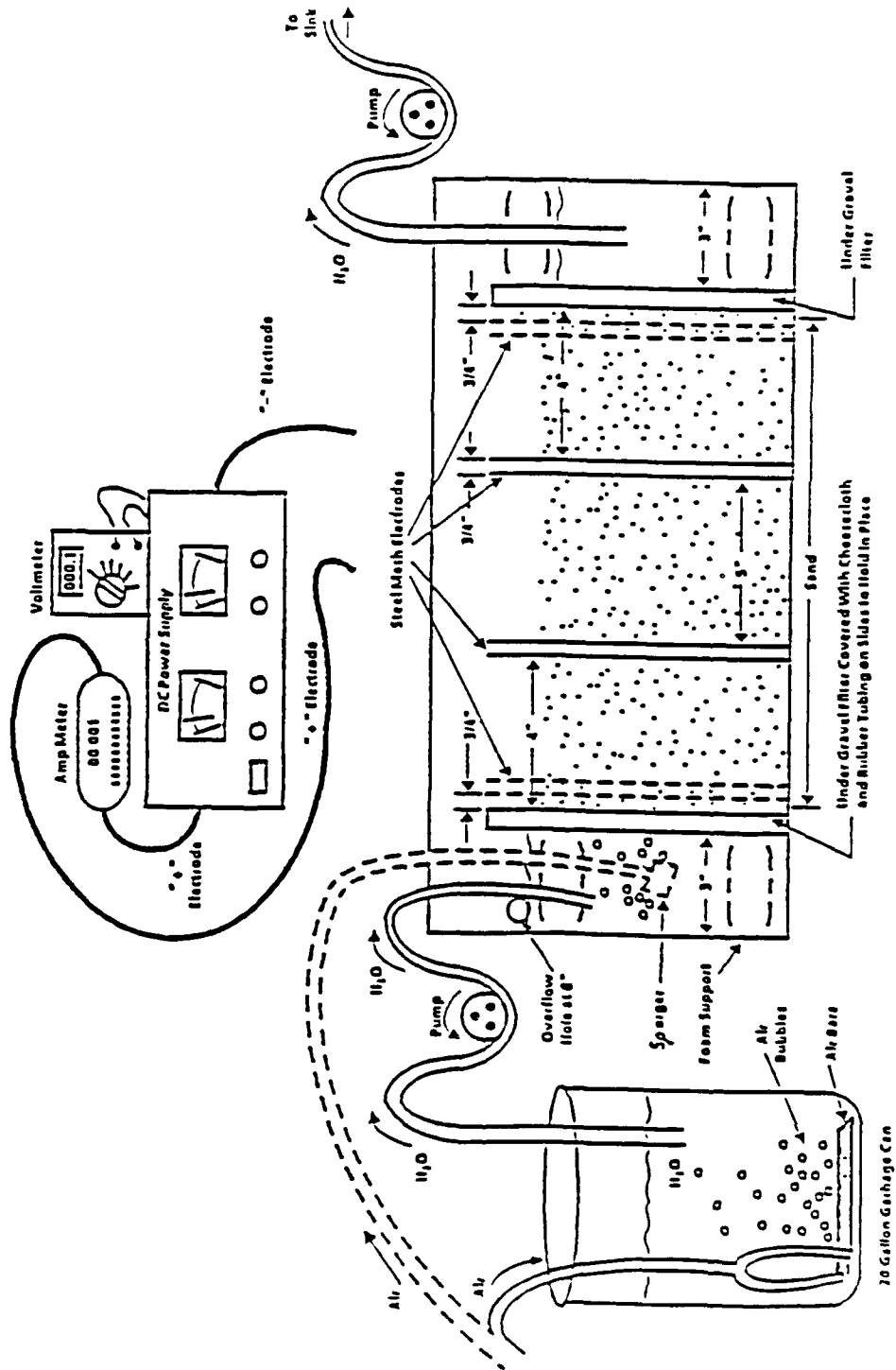


FIGURE 1. SCHEMATIC DRAWING OF SOIL CELL SET-UP USED FOR ELECTROCHEMICAL TESTS

Equipment and instruments used in the electrochemical tests included:

- Hewlett-Packard 6268 DC power supply
- Keithley 179-20A multimeter
- Fluke 77 multimeter
- Keithley 614 electrometer
- YSI 50 dissolved oxygen meter
- Orion 501 digital pH meter
- Jenway PCM 3 conductivity meter.

### **Experimental Procedure**

The soil sample was kept in the center chamber of the cell by two undergravel filters covered with cheesecloth and having pieces of rubber and tygon tubing along the glass walls to anchor the filter and to prevent leaks. Clear silicone sealant was used to seal the soil compartment.

Stainless steel mesh electrodes (10-inches x 10-inches) were placed in the cell and anchored in place by the soil. Eight electrodes were placed in the soil, but only two electrodes was used for each run.

Three vertical wells were made, about 4 inches apart, using tubings made of a sheet of plastic screen, to allow measurement of dissolved oxygen in the "groundwater."

Before each run, the 20-gallon plastic drum (garbage can) was filled with about 15 gallons of tap water and allowed to equilibrate to ambient temperature. Compressed air then was bubbled into the water for at least 20 hours. Typically the dissolved oxygen content did not increase beyond about 9 ppm after 20 hours of air bubbling. The water was then allowed to flow through the soil layer. A second hose, with a sparger at the end, pumped air into the left chamber of the cell. The additional air bubbling was used to ensure that the water entering the soil layer had maximum air (oxygen) saturation. An overflow hole was cut in the tank wall at a height of 8-inches to prevent "flooding" over the soil. Water was allowed to flow through the soil layer, but not over the top of the soil layer.

For the sand/bentonite mixture, it took about 2 hours for the water penetrate through the soil layer (about 13 inches) and accumulate to a height of 4 inches in the reservoir on

the other side of the soil layer (right chamber). For the Fallon soil, it took over 6 hours to achieve the same conditions.

When the height of the water in the exit chamber (right reservoir) reached 4 inches, electrical current was turned on and adjusted to the appropriate value for the run.

Measurements of the following properties were performed during each run: dissolved oxygen, pH, temperature, conductivity, overall voltage, current, and potential at electrodes.

## **RESULTS**

### **Results of Experiments with Sand/Bentonite Mixture**

Seven different tests were run with a sand/bentonite mixture as the soil in order to establish optimum operating conditions. Of particular concern were the values for the driving voltage and current that would result the highest dissolved oxygen content in soil. The range of voltages examined was 7.5 to 10.0 volts, which resulted a current range of approximately 50 to 100 mA. Another experimental variable examined in this series of tests with the sand/bentonite mixture was the distance between the anode and the cathode, which ranged from 3 inches to 13 inches.

The results of these tests can be summarized as follows:

- The average flow rate of water through the soil layer was about 65 ml/min
- A driving voltage of 10 V yielded an average 100 mA current flow between the electrodes
- At the 10 V/100 mA combination, the highest dissolved oxygen level was achieved: the dissolved oxygen content near the anode increased from the initial value of 9 ppm to 16 ppm in one half hour but did not increase further
- At the 10 V/100 mA combination, the dissolved oxygen content at the cathode decreased slightly, from the initial value of 9 ppm to 7 ppm
- The dissolved oxygen content at locations other than the anode area remained mostly unaffected by the electrolysis, maintaining the initial value of about 9 ppm



- No significant change in pH was observed in the groundwater
- The distance between electrodes did not affect the concentration of oxygen at the anode or the cathode.

Table 1 shows typical results obtained in these tests. The electrochemical parameters set for this particular set of results were: 10 V driving voltage, a nominal current value of 100 mA, 3-inch electrode spacing, and a 65 ml/min water flow rate. The anode was placed at about 2 inches from the inlet water chamber, and the cathode, in turn, was 3 inches away from the anode.

The results presented in Table 1 show that the dissolved oxygen content at the anode increased rapidly in the first 30 minutes, reaching 16 ppm. The slight decrease afterwards was not typical in other tests. Surprisingly, the oxygen content at the cathode did not decrease significantly; it remained at the value same as that of the inlet water (9 ppm) for at least 1 hour and then decreased to 7 ppm as the time increased. Also significant is the fact that the dissolved oxygen content at other locations (away from the electrodes) in the soil remained relatively unchanged. The acidity (pH) of the outlet water was about the same as that of the inlet water.

### **Results of Experiments with Fallon Soil**

For the tests with the soil sample from Fallon, Nevada, a the total current of 100 mA, which yielded most dissolved oxygen in the preliminary tests with sand/bentonite mixture, was used for all tests. The resulting voltage between the anode and the cathode varied depending on such factors as the distance between the electrodes and the degree of wetness of the soil (as affected by the height of the water in the outlet reservoir). At 100 mA current, the potential of the anode was about 1.5 V and that of the cathode about -1.2 V (potentials were measured against standard Calomel electrode).

Fallon soil was basically clay soil and thus the water flow rate through the soil layer in the test cell was extremely low. It was typically about 1 ml/min. To wet the soil initially, therefore, the soil was flooded from the top after it was loaded into the cell. Tests began after at least 4 inches of water was collected in the outlet water chamber.

TABLE 1. RESULTS OF A ELECTROLYSIS TEST WITH SAND/BENTONITE MIXTURE

Time (min.)	Flow Rate (ml/min)	Voltage (V)	Current (mA)	Oxygen at Inlet Water (ppm)	Oxygen at Anode (ppm)	Oxygen at Cathode (ppm)	Oxygen away from Electrodes (ppm)	Oxygen at Outlet Water (ppm)	pH Inlet Water	pH Outlet Water
0	63.6	10	128	9.5	9.4	9.2	9.2	8.2	8.05	8.11
30	64.4	10	123	9.6	15.9	9.3	8.4	8.2	8.05	8.06
60	64.4	10	119	9.5	16.1	9.0	8.5	8.2	8.04	8.11
90	65.0	10	116	9.5	15.7	7.8	8.8	8.2	8.07	8.10
120	64.8	10	114	9.4	15.6	7.1	8.7	8.2	8.03	8.07
150	64.8	10	112	9.4	15.5	7.0	8.6	8.2	8.07	8.09
180	64.8	10	110	9.4	15.2	7.0	8.6	8.2	8.05	8.06

(Temperature of the soil cell remained constant at 19C throughout the test.)

Three electrochemical tests were performed with Fallon soil. The results may be summarized as follows:

- Water flow rate through Fallon soil was about 1 ml/min
- Dissolved oxygen content dropped from about 9 ppm to about 2 ppm as soon as the air saturated water came in contact with the soil; oxygen is apparently consumed by the soil
- Dissolved oxygen content at locations away from the electrodes remained low throughout the test duration
- The highest dissolved oxygen content achieved at 100 mA current was 12 ppm in 22 hours
- Dissolved oxygen content at the cathode decreased slightly
- When the soil was not fully saturated, the pH at the anode decreased substantially whereas that at the cathode increased substantially.

Tables 2, 3, and 4 summarize the test results for each of the three runs made with Fallon soil. When the test was run for up to 22 hours, the dissolved oxygen content at the anode area reached 12.1 ppm (as shown in Table 3). The oxygen content at the cathode at this time was 1.3 ppm, which was much less than that at the anode. However, it should be noted that 1.3 ppm is not significantly lower than 1.7 ppm, the oxygen content at time zero. The most notable feature in the data shown in Table 3 is the significant decrease in pH at the anode.

Table 4 summarizes the results obtained in Run No.3. The experimental condition for this run were significantly different from those employed for Run No.1 and 2. In Run No. 3, the distance between the electrodes were shortened to 3 inches (as opposed to 13 inches for Run Nos. 1 and 2), and the soil layer was also shortened by four inches. These changes were made to enhance higher water flow through the soil layer. Because of these changes, the voltage required to drive 100 mA current was only about 4 volts. The oxygen content at the anode after 66 hours reached 9.4 ppm, a value lower than had been expected.

TABLE 2. RESULTS OF A ELECTROCHEMICAL TEST WITH FALLON SOIL (Run No. 1)

Time (hr.)	Voltage (V)	Current (mA)	Oxygen at Inlet (ppm)	Oxygen at Anode (ppm)	Oxygen at Cathode (ppm)	Oxygen away from Electrodes (ppm)	Oxygen at Outlet (ppm)	pH at Inlet	pH at Anode	pH at Cathode	pH at Outlet
0	0	0	7.0	1.7	1.6	1.7	2.7	6.4	7.4	7.4	7.2
1.0	8.86	99.6	7.1	6.2	1.5	1.3	2.0	6.8	3.4	7.5	7.5
2.0	9.17	100.5	7.0	9.5	1.5	1.4	1.9	7.0	3.4	7.6	7.4
3.0	9.33	100.0	7.0	10.7	1.5	1.6	1.7	7.1	3.4	7.5	7.4
4.0	9.59	100.2	6.9	9.7	1.5	1.5	1.8	7.3	2.5	7.8	7.6
5.0	9.83	100.8	7.0	9.7	1.3	1.3	1.4	7.2	4.6	7.3	8.1

(Electrode distance = 13 inches.)

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TABLE 3. RESULTS OF A ELECTROCHEMICAL TEST WITH FALLON SOIL (Run No. 2)

Time (hr.)	Voltage (V)	Current (mA)	Oxygen at Inlet (ppm)	Oxygen at Anode (ppm)	Oxygen at Cathode (ppm)	Oxygen away from Electrodes (ppm)	Oxygen at Outlet (ppm)	pH at Inlet	pH at Anode	pH at Cathode	pH at Outlet
0	0	0	7.0	1.7	1.7	1.7	3.0	7.5	7.4	7.9	7.5
3.5	13.51	100.1	7.0	9.4	1.7	1.7	3.0	7.5	6.4	7.9	8.5
5.5	13.41	101.5	7.0	10.8	2.9	1.8	3.2	7.3	3.8	7.3	8.6
11.5	13.41	103.7	7.0	10.2	0.9	0.7	2.6	7.4	2.6	7.2	8.4
22.0	13.36	103.4	7.4	12.1	1.0	1.3	n/a	7.3	1.6	8.6	n/a

(Electrode distance = 13 inches.)

TABLE 4. RESULTS OF A ELECTROCHEMICAL TEST WITH FALLON SOIL (Run No. 3)

Time (hr.)	Voltage (V)	Current (mA)	Oxygen at Inlet (ppm)	Oxygen at Anode (ppm)	Oxygen at Cathode (ppm)	Oxygen away from Electrodes (ppm)	Oxygen at Outlet (ppm)	pH at Inlet	pH at Anode	pH at Cathode	pH at Outlet
0	0	0	8.0	3.9	3.9	4.1	4.8	7.6	8.6	8.6	8.3
1.0	3.94	104.0	7.4	4.0	3.5	3.5	5.0	7.9	8.8	8.7	8.4
2.5	3.71	100.0	7.6	3.9	3.5	3.4	4.6	8.0	8.7	8.7	8.4
5.0	3.71	96.5	8.0	4.8	2.7	3.4	5.4	8.0	8.6	8.8	8.5
15.5	3.78	94.0	8.5	8.0	4.6	4.3	7.0	8.0	8.7	8.8	8.5
31.0	3.89	95.4	8.5	9.0	3.3	3.1	6.5	8.0	7.8	9.0	8.4
45.0	3.99	94.0	8.9	7.4	2.2	3.4	6.3	8.0	7.4	9.8	8.7
54.0	4.09	100.0	9.4	7.6	3.0	4.0	6.2	8.0	7.2	10.0	10.8
66.0	4.09	100.0	9.4	8.0	4.1	4.0	6.9	8.0	6.9	11.4	10.1

(Electrode distance = 13 inches.)

### Effect of Electrolytic Oxygen Production on Microorganisms

As was stated earlier, two sets of soil samples were taken from the soil cell for counting of soil microorganisms—one set of soil samples before the application of electrolytic oxygen generation and another set afterward. The Fallon soil used in Run No. 3 was used for the counting. Soil samples were taken from the anode area and from the cathode area. The dissolved oxygen contents of the two areas before and after the electrolysis run were as follows:

Anode area:	4.8 ppm before	8.0 ppm after 66 hours
Cathode area:	4.8 ppm before	4.1 ppm after 66 hours

### Media, Sample Preparation and Incubation Conditions

Heterotrophic, eutrophic bacteria grow on a variety of organic carbon sources unless the carbon sources are at very low concentrations. Nutrient agar was chosen as a good medium for growth of these organisms. Oligotrophic bacteria, which grow on low concentrations of organic carbon sources, were grown on diluted nutrient agar (solid agar support was maintained at 1.5 percent). Filamentous fungi can be counted on a variety of media including, for example, rose-bengal or Sabouraud Maltose Agar. Sabouraud Maltose Agar was used for this study based on availability. Actinomyces can be grown on various media such as starch-casein or a relatively new, commercially available Actinomyces Isolation Agar. Actinomyces Isolation Agar was used in this study.

Soil samples were obtained by taking cores (using pipettes with the tips removed). Subsamples (1 g wet weight) were serially diluted in sterile distilled water. The dilutions were chosen based on population densities reported by Arthur and Frea (1988). The samples were vortexed vigorously between dilutions. The spread-plate method was used in the counting of all organisms, using 5 replicate plates per dilution. All incubations were carried out at 25°C. Following is a summary of media, dilutions employed in the plating procedure and incubation periods:

Population Incubation	Medium	Dilutions	Incubation Period
Heterotrophic bacteria	Nutrient Agar	$10^{-1}$ to $10^{-4}$	3 days
Oligotrophic bacteria	0.01X Nutrient Agar	$10^{-1}$ to $10^{-4}$	21 days
Filamentous fungi	Sabouraud Maltose Agar	$10^{-0}$ to $10^{-3}$	3 days
Actinomyces	Actinomyces Isolat. Agar	$10^{-1}$ to $10^{-4}$	15 days

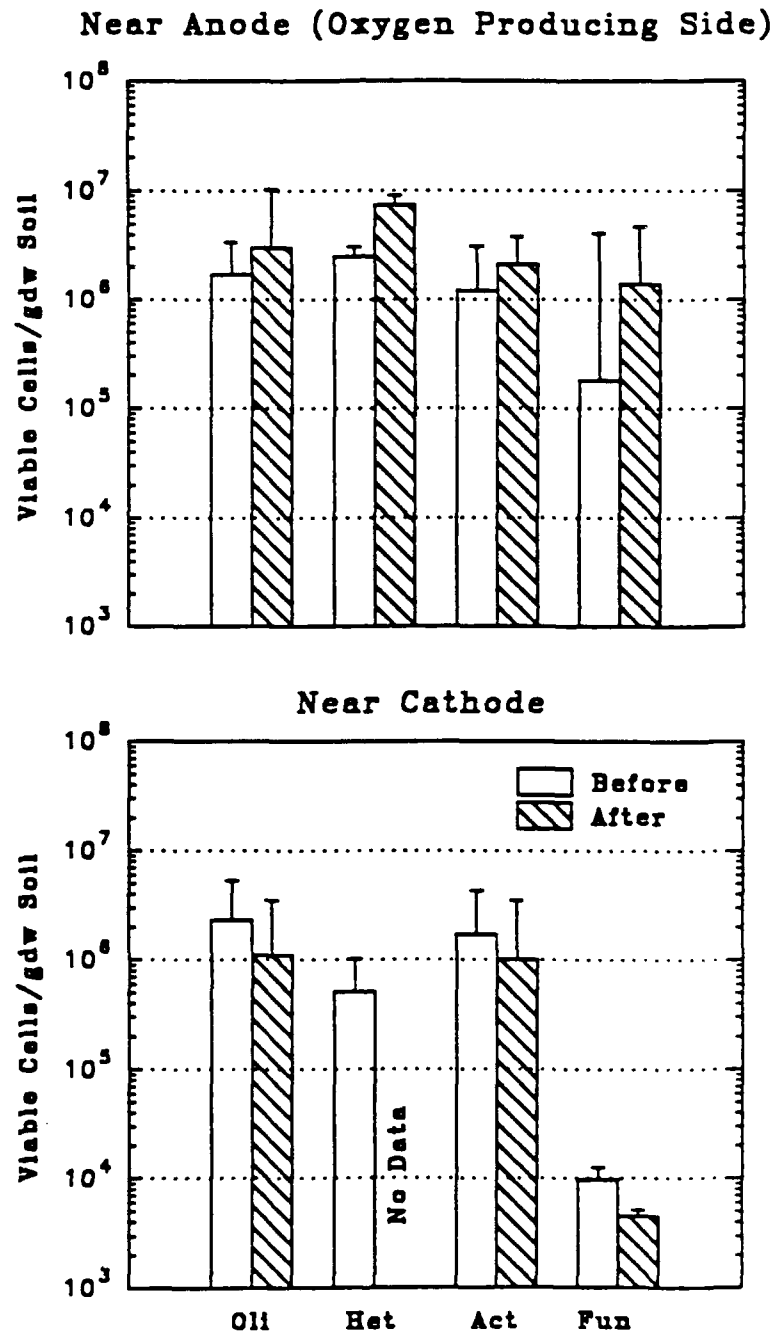
### Soil Dry Weights

Soil dry weights were determined by placing 1 g quantities of soil samples in pre-weighed aluminum pans and drying at 110°C for 24 hours. Counts were calculated based on gram dry weights (gdw) of soils.

### Results

Results of the microorganism counts are shown in Figure 2. The bars represent standard deviations. Oligotrophic microbes were at  $2 \times 10^6$ /gdw soil before electrolysis, both near the anode and near the cathode. Similar population densities were observed for the heterotrophs and organisms growing on Actinomyces Isolation Agar. Fungi were 1 to 2 order of magnitude lower in concentration than were the other organisms. After electrolysis, the populations appeared to shift slightly such that growth occurred at the anode, and a slight decline in the populations occurred at the cathode. This was an expected finding, since oxygen which supports growth was generated at the anode region. The slight decline at the cathode may have been due to a lowering in pH or some other reaction. No data were available for heterotrophic population levels after electrolysis due to a technical error.

Unexpectedly, microscopic examination of colonies from the Sabouraud Maltose plates in this initial study revealed that most of the organisms were yeasts or bacteria, rather than fungi. In future work, streptomycin (1 ml of a 3 percent solution per liter of medium) should be included in the medium to suppress bacterial growth.



**FIGURE 2. EFFECT OF ELECTROLYSIS ON SOIL MICROORGANISMS POPULATION**



### CONCLUSIONS

Based on the results obtained in this study, the following conclusions were made:

- Long, sustained supply of oxygen to soil microorganism appears to be feasible by in-situ generation of oxygen by electrolysis. The highest concentration of dissolved oxygen achieved in this limited study was 16 ppm, but it appears that a higher current/voltage combination should allow higher concentrations.
- The highest concentration of dissolved oxygen obtained with Fallon soil was 12 ppm. The rate of increase in dissolved oxygen content in this soil was lower than that for the sand/bentonite mixture. Oxygen contents in areas away from the anode remained low, even when the oxygen content at the anode increased to ten times its initial concentration.
- The population of soil microorganism increased at the anode area but decreased slightly at the cathode area. The increase at the anode corresponded well with the increase in dissolved oxygen content. The decrease at the cathode may have been due to the increase in pH. More tests are needed to deduce firm conclusions.

### RECOMMENDATIONS

It is recommended that further development be carried out for a thorough investigation of experimental parameters that affect the electrolysis process. Large scale tests should then follow to simulate field conditions in a controlled environment.

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